Functional Properties of Soybean and Lupin Protein Concentrates Produced by Ultrafiltration-Diafiltration

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ABSTRACT: Ultrafiltration followed by diafiltration (UF-DF) was evaluated for the production of protein products from partially defatted soybean meal or undefatted lupin (*Lupinus albus* L.2043N) meal. This study determined the effects of UF-DF on functional properties of the extracted proteins and compared the results with those of protein prepared by acid-precipitation (AP). UF-DF produced only protein concentrates (73% crude protein, dry basis, db), while AP produced protein isolates (about 90% crude protein, db). Soybean protein produced by UF-DF showed markedly higher values for solubilities up to pH 7.0, surface hydrophobicity index, emulsion activity index, and foaming capacity than did the AP soybean protein. UF-DF soy protein was also the most heat-stable among all protein samples tested. With lupin proteins, only the surface hydrophobicity and emulsion activity indices were significantly improved by using UF-DF. UF-DF generally had no adverse effects on, and in most cases even improved, the functional properties of soy protein concentrate produced by this method. UF-DF did not produce a comparable improvement in functional properties of lupin proteins as it did for soybean protein.

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KEY WORDS: Diafiltration, functional properties, lupin, protein concentrate, protein isolate, soybean, ultrafiltration.

The Clean Air Act, which limits emissions of volatile organic compounds and other hazardous air pollutants, has spurred the utilization of mechanical extraction processes in the oilseed industry (1). One such solvent-free process is extrusion-expelling (E-E), a simple and relatively inexpensive operation (2) that has produced soybean oil of such quality that only minimal refining will be required (3). The resulting meal has higher oil content than what is found in meal produced from traditional solvent extraction (2), but E-E meal is considered as a higher energy source for livestock feed (1). Because E-E does not use desolventizing and drying, the meal is also presumed to have better functional properties, which would make it possible to use E-E meal for the production of the higher-value flours, concentrates, or isolates. Heywood *et al*. (4) prepared low-fat soy flours from E-E meal and found them to possess good solubilities (at pH values less than and greater than 4), emulsifying and foaming properties, and water- and fat-binding capacities. No study has yet been reported on the preparation and quality of concentrates or isolates from E-E meal.

The present study explores ultrafiltration (UF) as a means of producing protein concentrates or isolates from E-E soybean meal. UF is a pressure-driven membrane process that has gained wide acceptance in protein bioseparations (5). It has a broad variety of applications, but it is used mainly for concentration, desalting, clarification, and fractionation (5). Sessa (6) recently produced peroxidase and Bowman–Birk inhibitor concentrates from soybean hulls by using UF, followed by discontinuous diafiltration (DF), a membrane-based method that removes low M.W. solutes such as salts while continuously replacing the solvent lost with the permeate (5). Both peroxidase and Bowman–Birk inhibitor are valuable products that have potential medical uses.

Our investigation of a UF-based approach to producing protein concentrates or isolates also included lupin seed proteins. Lupin (genus *Lupinus*) is a legume that contains approximately 5–20% crude oil, 36–52% crude protein, 30–40% crude fiber, and 4% ash in the whole seed (7,8). The FA composition of lipids in lupin seeds was found to be remarkably similar to that of soybean, while the protein was observed to contain low amounts of sulfur-containing amino acids and a good balance of essential amino acids (8). Lupin seed proteins were highly soluble at pH >5.5, and showed good water- and fat-binding capacities, foaming capacity, and emulsifying ability (9). Despite these attractive qualities, lupin has had limited utilization because of its alkaloid levels and low agronomic yield (7). Breeding programs in Australia, Chile, and the United States have now overcome these problems, and there is renewed interest in developing lupin as a protein or fiber source for both human foods and animal feeds (7).

The main goal of our research was to evaluate the performance of UF followed by discontinuous diafiltration (UF-DF) as an alternative approach for the production of protein products. The present study was conducted to determine the effects of UF-DF on functional properties of extracted proteins from partially defatted soybean meal or undefatted lupin (*Lupinus albus* L.2043N) meal and compare the results with those of proteins prepared by acid-precipitation (AP).

EXPERIMENTAL PROCEDURES

Starting materials. Extruded-expelled, partially defatted soybean meal, with a 66.5% protein dispersibility index (PDI),

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was provided by Nutriant (Cedar Falls, IA). Lupin (*L. albus* L.2043N) seeds were donated by Gene Aksland (Resource Seeds Inc., Gilroy, CA). Seeds were hand-dissected and dehulled and then ground at room temperature in a disc mill (Model S.500; GlenMills Inc., Clifton, NJ) by using the "Coarse" setting in the first pass and the "Fine" setting in the second pass. Ground lupin samples were passed through 30-mesh sieve to obtain whole meal. Grinding was repeated as needed for coarse particles retained on the 30-mesh sieve. Soybean and lupin meals were stored in sealed polyethylene bags at room temperature until use.

Proximate analyses. Moisture, crude oil, crude protein (N × 6.25), crude fiber, and ash contents of the meals were determined by using AOCS standard methods Ba 2a-38, Ba 3-38, Ba 4e-93, Ba 6-84, and Ba 5a-49, respectively (10). AOCS standard methods Ba 2a-38 and Ba 4e-93 were also used to determine moisture and crude protein contents, respectively, of the concentrates or isolates (10).

Production of protein extracts. (i) UF-DF. Protein was extracted and recovered from the meal by adapting the method of Sessa (6) (Fig. 1). Fifty grams of meal was homogenized with water (1 g/34 mL ratio) at 5000 rpm for 15 min at endogenous pH (6.6) and room temperature (25°C) by using a mixer/emulsifier (Ross Model HSM100LC; Charles Ross and Son Co., Hauppauge, NY) equipped with a disintegrator head. The mixture was centrifuged at 18°C and 15,344 × *g* for 25 min and then filtered through Whatman no. 5 paper. The filtrate was set aside, while the solids were rinsed with water into the homogenizer beaker (total rinse volume = 1600 mL). The mixture was homogenized, centrifuged, and filtered as described previously. The solids were discarded. Filtrates from the two extractions were pooled, and the volume was adjusted to 4 L with water. The filtrate was subjected to UF in a Pall Centramate system equipped with 5 kDa MWCO polyether sulfone membrane and operated at a flux rate of 2.7 L/h. Inlet and outlet pressure ranges were 21–25 and 27–34 psi, respectively. When *ca.* 3400 mL of permeate was collected, 2 L of water was added to the retentate sample, which was concentrated again to a final volume of about 600 mL. The copious volume of permeate was discarded, while the concentrated retentate was freeze-dried to obtain the protein concentrate or isolate.

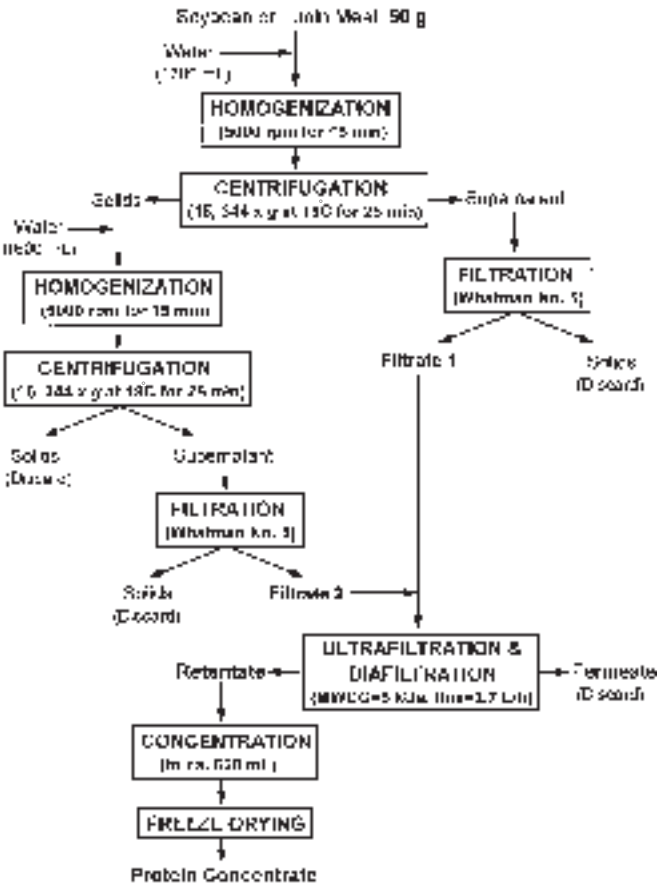

FIG. 1. Procedure for the production of soy or lupin protein concentrate using ultrafiltration-diafiltration (UF-DF).

(ii) Acid-precipitation (AP). The procedure for recovering proteins was adapted from the method of Thanh and Shibasaki (11). Fifty grams of meal was homogenized with 0.03 M Tris-HCl (1 g/20 mL ratio) at 5000 rpm for 15 min by using the Ross mixer/emulsifier. The mixture was centrifuged at 2–5°C and 15,344 × *g* for 20 min. Solids were discarded. The pH of the supernatant was adjusted to 4.5 by addition of 1 N HCl, after which it was centrifuged as described earlier. Solubles were discarded, while the precipitated protein was redissolved by neutralization with 2 N NaOH and then centrifuged at room temperature and 15,344 × *g* for 20 min. The supernatant was desalted by dialyzing in 1000 MWCO casing, and the extract was then freeze-dried to recover the concentrate or isolate.

SDS-PAGE. SDS-PAGE was done according to the method of Sessa and Wolf (12). Dialyzed and freeze-dried protein extracts were weighed out to provide 1–2.5 mg protein/mL in 500 μL of sample buffer [0.055 M Tris-HCl (pH 6.8), 2.0% SDS, 7.0% glycerol, 4.3% β-mercaptoethanol, and 5 M urea], then heated in a boiling-water bath for 5 min. Protein samples (15 μL) were loaded onto 14% acrylamide mini-gel, which had a 32:1 ratio of acrylamide/AcrylAide (FMC BioProducts, Rockland, ME) and was bonded to GelBond PAG film (FMC BioProducts). Bio-Rad (Bio-Rad Laboratories, Hercules, CA) prestained broad range SDS-PAGE protein standards (6.5–200.0 kDa) were included in the gel. Electrophoresis was done in a Bio-Rad Mini-PROTEAN II unit under the conditions described by Sessa and Wolf (12).

Functionality tests. (i) Solubility, foaming properties, and heat coagulability. These properties were evaluated by following precisely the procedures described by Myers *et al.* (13), which were modifications of the methods reported by Balmaceda *et al.* (14).

(ii) Emulsifying properties. The turbidimetric method of Pearce and Kinsella (15) as performed by Wu *et al.* (16) was used to determine emulsification activity index (EAI, in m^2/g) and emulsion stability index (ESI, in min). Homogenization was done on mixtures of 2 mL corn oil and 6 mL sample solutions (1 mg protein/mL) by using a handheld homogenizer

(Model 1000, Omni International, Waterbury, CT) operated at high setting (20,000 rpm) for 1 min. All other steps were done as described by Wu *et al.* (16).

(iii) Surface hydrophobicity. Surface hydrophobicity indices (S_o) of soluble proteins in our extracts were determined at neutral pH by following the method of Sorgentini *et al.* (17), which used 8.0 mM 8-anilino-1-naphthalene sulfonate (ANS) as fluorescence probe. Supernatants from sample dispersions (1 mg protein/mL) were diluted with 0.01 M phosphate buffer (pH 7) to provide 1:2, 1:5, 1:10, and 1:100 concentrations of the starting protein content. Fluorescence intensities (FI) were measured by a Varian Cary Eclipse Fluorescence Spectrophotometer (Walnut Creek, CA) at wavelengths of 410 nm (excitation) and 485 nm (emission). FI values were plotted against protein concentrations to determine S_o, which corresponded to the initial slope of the graph as calculated by linear regression.

Statistical analyses. Statistical analyses were performed by using the SAS® Systems for Windows software (SAS Institute Inc., Cary, NC). ANOVA and Duncan's Multiple Range tests were performed on duplicate replications of data to determine significant differences among the treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate composition of starting soybean and lupin meals. The proximate composition of E-E soybean meal (Table 1) was similar to that of commercially prepared soybean meal, except that its crude oil content was 10 times greater. The composition data we obtained were almost identical to what Heywood *et al.* (4) reported for the "high" group of low-fat soy flour they produced from E-E meal. The lupin meal had crude fat and ash contents that were similar to the10.0% oil and 4.0% ash reported by Mohamed and Rayas-Duarte (7), but its protein content was greater (46% vs. 38%). The crude fiber content of our lupin meal, however, was significantly lower than the typical 30–40% fiber in lupin (7) because the seeds we used were dehulled. High fiber content in lupin is concentrated in the hull (8).

TABLE 1
Proximate Composition of Partially Defatted Soybean Meal and Undefatted, Dehulled Lupin Meal Before Protein Extraction

Meal source	Moisture content (%)	Crude protein[a] (% db)	Crude fat (% db)	Crude fiber (% db)	Ash (% db)
Soybean	6.1	48.9	11.1	2.2	6.4
Lupin	7.2	46.4	12.2	1.3	3.7

[a]Dumas N × 6.25. db, dry beans.

Protein contents of soybean and lupin meal extracts. Freeze-dried soybean and lupin protein extracts produced by acid precipitation had significantly greater crude protein contents (Table 2). Lupin protein met the industry's standards for isolates, whereas a near-isolate was recovered from the soybean extract. Freeze-dried protein extracts recovered from UF-DF retentates contained about 70% (db) crude protein (Table 2), which classified them as concentrates according to industry protein standards. The oil that was initially present in both meals (determined to be 11% in soybean and 12% in lupin) may have caused some fouling in the membrane, as indicated by a reduction in flux near the end of the UF-DF process. Fouling may have prevented the efficient removal of nonproteinaceous components, thereby leading to lower crude protein contents of the products. Residual oil contents were determined to be 6.6 and 3.5% (db) in lupin and soybean protein concentrates, respectively. These correspond to 46 and 67% reductions in lipid contents of lupin and soybean meals, respectively, that were subjected to UF-DF.

Electrophoresis results. Profiles for all the soybean and lupin protein extracts (Fig. 2, lanes 4–7) showed protein bands just below the stacking gel wells, which indicated protein aggregation. Aggregate formation is a typical result of protein denaturation. Band patterns for the soy protein concentrates (lanes 4 and 5) were compared with those of 7S and 11S soy proteins. Protein bands for partially purified soy 7S with some 11S contaminant (lane 2) were between 45.0 and 66.2 kDa and designated α′, α, and β conglycinin (18). Soy 11S (lane 3) was composed of four protein bands: A, which resolved just above 45.0 kDa; A_{1a}, A_{1b}, and A_2, between 31.0 and 45.0 kDa; and, B, with MW corresponding to 21.5 kDa. All of the soy 7S and 11S protein bands were evident in the soy protein concentrates produced by acid precipitation (lane 4) and from UF-DF retentate (lane 5).

Protein bands for acid-precipitated lupin protein isolate (Fig. 2, lane 6) were not as clearly defined as those of lupin protein concentrate from UF-DF retentate (lane 7) and showed notable streaking in the gel pattern, which indicated that the protein had undergone significant denaturation. Both lupin protein products, though, were made up of a series of subunits

TABLE 2
Crude Protein Contents and Selected Functional Properties of Soybean and Lupin Protein Extracts Produced By Acid Precipitation (AP) or Ultrafiltration-Diafiltration (UF-DF)[a]

Protein source/method	Crude protein % db	Functional properties[b]					
		S_o	FC mL	FS % foam left	EAI m^2/g	ESI min	HC % protein lost
Soybean, AP	86.1 ± 0.4[b]	529.5 ± 21.7[b]	131 ± 7[a]	95.0 ± 1.8[a]	56.0 ± 3.3[bc]	15.0 ± 1.5[a]	24.5 ± 0.4[c]
Soybean, retentate, UF-DF	72.2 ± 0.5[c]	844.3 ± 24.6[a]	144 ± 3[a]	77.4 ± 3.0[b]	98.7 ± 4.2[a]	15.0 ± 0.2[a]	9.3 ± 1.9[d]
Lupin, AP	96.3 ± 0.5[b]	301.9 ± 0.6[d]	104 ± 12[ab]	16.8 ± 1.4[c]	45.4 ± 5.3[c]	23.4 ± 4.1[a]	48.3 ± 1.9[b]
Lupin, retentate, UF-DF	73.2 ± 0.9[c]	429.2 ± 16.4[c]	98 ± 3[b]	2.6 ±0.8[d]	71.5 ± 5.1[b]	25.5 ± 6.0[a]	58.8 ± 1.3[a]

[a]Values are means ± SD of duplicate determinations. Means within columns followed by different letters are significantly different ($P < 0.05$).
[b]S_o, surface hydrophobicity index; FC, foaming capacity; FS, foam stability; EAI, emulsion activity index; ESI, emulsion stability index; HC, heat coagulability.

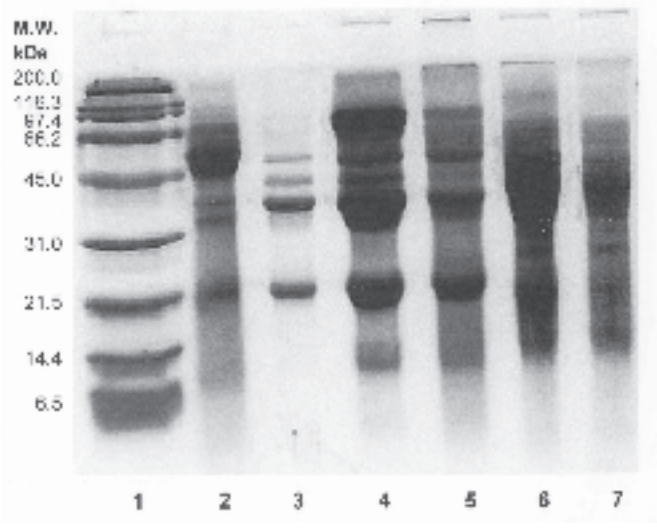

FIG. 2. SDS-PAGE profiles of soybean and lupin protein extracts produced by acid precipitation (AP) or UF-DF: (1) M.W. standards; (2) soybean, 7S fraction; (3) soybean, 11S fraction; (4) soybean, AP; (5) soybean, UF-DF; (6) lupin, AP; and (7) lupin, UF-DF. For other abbreviation see Figure 1.

that were heavily concentrated between 31.0 and 66.2 kDa. There were protein bands that resolved at >97.4 kDa, moderately concentrated bands that were estimated to be between 18 and 21.5 kDa, and less concentrated bands between 21.5 and 31.0 kDa. These profiles were similar to those of lupin seed flour and protein concentrate reported by Sathe *et al.* (9). Quantitative differences among the subunits for soy or lupin concentrates and isolates were not determined in this study.

Solubility profiles. The solubility profiles (Fig. 3) show that almost all the protein products appeared to have reached their maximum solubilities around pH 7. The exception was AP soybean protein, which showed increasing solubility with pH until it was 100% soluble at pH 10. While the presence of alkali usually improves solubility, caution must be taken in interpreting this result because high solubility also may indicate extensive proteolysis and disaggregation (19).

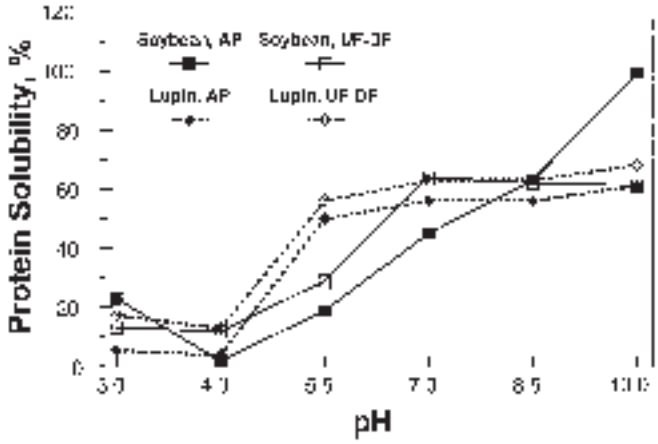

FIG. 3. Solubility profiles of soybean and lupin protein extracts produced by AP or UF-DF. See Figures 1 and 2 for abbreviations.

Up to pH 7, UF-DF soybean protein concentrates (SPC) were significantly more soluble than the AP soybean proteins (65% vs. 45%), possibly owing to less harsh conditions in UF-DF. The 65% soluble protein is also considerably greater than the ≤13% and 49% average solubilities at pH 7 for several commercially available SPC and flour, respectively, which were used in another study by Hojilla-Evangelista (unpublished data). The solubility we obtained at pH 7 was likewise markedly higher than the values reported by Heywood *et al.* (4): 15–45% for various low-fat soy flours that they produced from E-E meals.

Solubilities of lupin protein products were lowest at pH 4, increased significantly at pH 5.5, and leveled off beyond pH 7 (Fig. 3). Both profiles, until pH 8.5, were similar to the results reported by Sathe *et al.* (9). UF-DF lupin protein concentrates (LPC) were more soluble than acid-precipitated lupin protein isolates (LPI) at all pH levels tested (Fig. 3). LPC had 63% soluble proteins at pH 7, which increased slightly to 68% at pH 10. Solubility of LPC was very similar to that of the SPC and markedly greater than those observed for commercial soybean flour and concentrates as discussed in the preceding section. However, the solubilities we obtained for lupin proteins were far less than those reported by Ruiz and Hove (20), who observed 90% solubility at pH >8.

The profiles showed that UF-DF produced protein concentrates that were generally more soluble than those produced by acid precipitation. The 65% solubilities at neutral pH of both UF-DF SPC and LPC indicate that these protein products may find some applications in most food systems.

S_o. S_o is a measure of the degree of exposure of hydrophobic regions of protein molecules, and high values are indicative of unaggregated proteins. The S_o determined at neutral pH for UF-DF SPC was 60% greater than that of AP soybean protein (Table 2). This finding suggests that in SPC produced by UF-DF, the proteins are better dispersed, which allows access to hydrophobic regions and lessens aggregation. This result may also explain why greater solubility was observed for UF-DF SPC. The outcome of the comparison of S_o values for the lupin protein products was similar to what was observed for soybean proteins. S_o for UF-DF LPC was 1.4 times greater than that of the AP LPI. However, S_o data for both lupin protein products were considerably less than those of the soybean proteins, implying a greater presence of aggregated proteins or degree of denaturation. This assumption is supported by the streaked appearance of the lupin protein bands, especially those of the isolate, in electrophoresis gel (Fig. 2).

Foaming properties. One percent solution of UF-DF SPC produced the greatest foam volume (Table 2), but it was not different from that produced by a similar solution of AP SPC. Foams produced by AP SPC were very stable, with 95% of foam volume retained after standing for 15 min. Foam stability of UF-DF SPC was not as high, but it was notable that such a level of stability was obtained, considering that UF-DF SPC still contained some oil (3.5% db), which is an effective defoamer. Lupin protein products produced nearly identical foam volumes of about 100 mL (Table 2), but their foam volumes

were less than those produced by the soy proteins. This result was similar to that observed by Sathe *et al.* (9) in their study on lupin flour and concentrate. Lupin protein foams contained large bubbles that were not uniformly distributed and collapsed rapidly shortly after formation, unlike the SPC foams. The instability of LPC foam may be attributed to the defoaming action of residual oil in LPC (6% db). Another possible explanation is the influence of pH on foaming properties of lupin seed protein concentrates. Sathe *et al.* (9) determined maximum foam stability for LPC at pH 4 (isoelectric region) and reported that stability progressively declined as the pH became more alkaline. In our study, foaming properties were evaluated at pH 7, thereby producing extremely unstable foams for lupin proteins.

Emulsifying properties. EAI measures the area of oil–water interface stabilized by a unit weight of protein (16). The EAI values for UF-DF SPC and LPC were markedly greater than those of their AP counterparts (Table 2), indicating that UF-DF SPC and LPC had much better emulsifying capabilities. ESI measures an emulsion's ability to resist breakdown (16). Higher ESI values indicate more stable emulsions. ESI values for all samples, ranging15–26 min, were fairly low and not statistically different ($P > 0.05$). ESI for UF-DF SPC was similar to that determined by Heywood *et al.* (4) for low-fat soybean flour produced from E-E meal. ESI for UF-DF LPC supports the previous finding by Sathe *et al.* (9) that emulsion stability for LPC was considered to be poor.

Heat coagulability. SPC produced by UF-DF was the most heat-stable among the products tested, showing only 9% loss in protein solubility after heating in 100°C water-bath for 30 min (Table 2). The protein loss observed for UF-DF SPC was markedly less than the 36% reported by Myers *et al.* (13) for acid-washed SPC. Both LPI and LPC were markedly more heat-labile than the soybean proteins, with 50–60% loss in solubility after heating.

UF-DF demonstrated strong viability as an alternative approach to producing protein concentrates from partially defatted E-E soybean meal. SPC from UF-DF retentate showed improved functional properties, such as solubilities up to pH 7, surface hydrophobicity, foaming capacity, emulsifying activity, and heat stability. These properties are useful for both food and nonfood applications. Surface hydrophobicity and emulsifying activity indices were the only functional properties improved by UF-DF in lupin protein concentrates.

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